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Atomic Structures Reveal a Unique Molecular Mechanism for Initiating TRAF6 Signaling

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A family of proteins called TRAF is key to many cellular communication pathways. Although the six members of this family have certain structural and functional similarities, their differences are still being investigated. A team of scientists led by Hao Wu, an x-ray crystallographer at Cornell University's Weill Medical College in New York City, has now determined with high resolution the atomic structures of TRAF6 and its derivative structures. This finding is a continuation of four years of research into the elucidation of TRAF structures by Wu's team. Crucial structural and functional insights are presented via rigorous comparative analyses of these various TRAF structures.

Cells talk to each other by exchanging molecules called messengers, such as growth factors (diffusible molecules affecting cellular growth), hormones (secreted in the blood by ductless glands) and neurotransmitters (molecules released between two neurons or a neuron and a muscle or a gland). On the cell surfaces, molecules called receptors bind to the exchanged molecules, activating proteins inside the cell. These proteins act together in an orderly fashion, defining what is called a signaling pathway.

One of the most potent cellular messengers, called tumor necrosis factor (TNF), binds to the TNF receptor (TNFR) located on the membrane surface of target cells and induces a wide range of cellular effects such as cell survival, proliferation, differentiation and death. A family of proteins that are major mediators of these effects is called TNF receptor associated factors (TRAFs).

To date, the TRAF family consists of six members that share certain com-

mon structural and functional domains. TRAFs can either directly interact with ligated TNFRs or indirectly form complexes with receptors binding to other signaling proteins. The binding of TRAFs to ligated TNFRs recruits more proteins, leading to multiprotein intracellular complexes that activate members of the families of proteins called necrosis-factor-kappa-B (NF- κ -B) and activator protein 1 (AP-1). NF- κ -B promotes the expression of genes involved in inflammatory and anti-apoptotic responses, while AP-1 can induce stress responses and cell death.

The signaling pathway of TRAF6 may be defined as follows. TRAF6 interacts with two members of the TNF receptor family called clusters of differentiation 40 (CD40) and TNF-related activation-induced cytokine receptor (TRANCE-R). TRAF6 also participates in the interleukin-1 receptor (IL1-R)/Toll-like receptor (TLR) pathway by coupling to a protein called interleukin-1 receptor-associated kinase (IRAK).

We have recently revealed significant differences in the way receptors bind to TRAF6 and TRAF2 (**Figure 1**). First, a receptor's peptide chain bound to TRAF6 deviates directionally from that bound to TRAF2 by about 40 degrees, so the reactive groups of the TRAF6-bound peptide latch onto pockets on TRAF6 that are very different than those on TRAF2. Second, the TRAF6-bound peptides assume extended β -conformations, unlike the polyproline II (PPII) helix conformation adopted by the core region of TRAF2-binding peptides. Third, the TRAF6-bound peptides make more extensive



Some of the authors of the study (from left to right): Hao Wu, Hong Ye and Oki Dzivenu.

main-chain hydrogen bonds with TRAF6.

Excessive activation of TRANCE-R can lead to significant resorption of bone, causing severe diseases such as osteoporosis and cancer-induced bone lesions. By using TRAF6-bind-

ing “decoy” peptides that we designed based on our structural studies and in collaboration with Bryant Darney at the MD Anderson Cancer Center in Houston, Texas, we have observed inhibition of bone resorption in cells derived from mice (**Figure 2**).

We have identified a universal structural mechanism by which TRAF6 participates in adaptive immunity, innate immunity and bone homeostasis. Our results may ultimately lead to drugs that modulate TRAF6-mediated signaling processes.

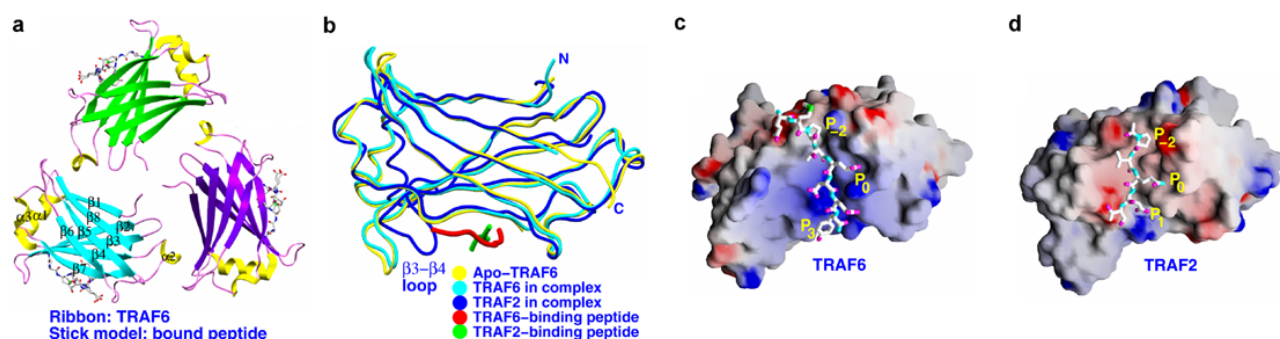


Figure 1. TRAF6 structures. (a), Ribbon diagram of the complex TRAF6 with TRANCE-R, shown as a trimeric model. (b), Worm representation of superimposed TRAF6 and TRAF2 structures. (c), Surface representation of TRAF6 and the bound TRANCE-R peptide. (d), Surface representation of TRAF2 and the bound core CD40 peptide.

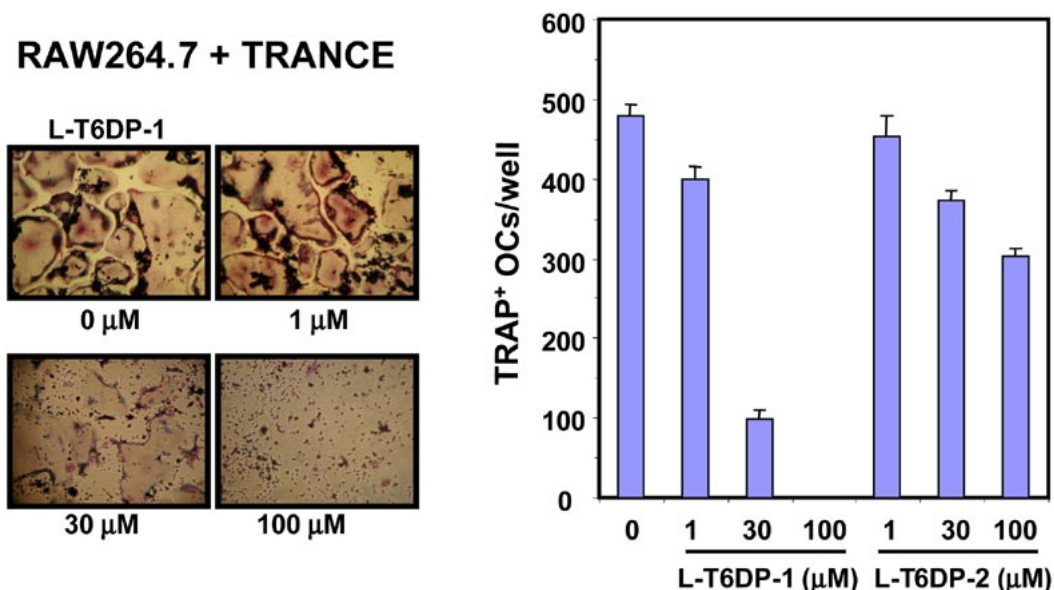


Figure 2. Co-treatment of RAW264.7 cells with TRAF6-binding “decoy” peptides known here as L-T6DP-1 and L-T6DP-2 caused a dose-dependent decrease of osteoclasts – cell responsible for bone resorption – which are tartrate-resistant acid phosphatase-positive (TRAP+) and multinucleated.